

Penetration of the Radial Hemal and Perihemal Systems of *Linckia laevigata* (Asteroidea) by the Proboscis of *Thyca crystallina*, an Ectoparasitic Gastropod

by

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Abstract. The proboscis of female *Thyca crystallina* (Mollusca) penetrates the body wall of *Linckia laevigata* (Echinodermata) and terminates in the middle of the ambulacral ridge where it opens within the perihemal sinus near the radial hemal strand. The proboscis does not penetrate the perivisceral coelom of its host, nor can it be withdrawn because the attachment disc of the adult snail is fused to the epidermis of the host. Except for a partial alteration in size and texture of a few ambulacral ossicles, and loss or reduction in size of the host's ampullae at the site of infection, other host organs and tissues are relatively unaffected by the penetration of the proboscis. Presumably *T. crystallina* obtains nutrients from the hemal-perihemal systems of its asteroid host.

INTRODUCTION

Because females of the ectoparasitic snail *Thyca crystallina* (Gould, 1846) are fused to their hosts, *Linckia laevigata* (Linnaeus), lack mouth parts, and have reduced digestive and enlarged salivary glands, many authors have concluded that they must be utilizing nutrients not requiring complex digestion (SARASIN & SARASIN, 1887; KÜENTHAL, 1897; KOEHLER & VANEY, 1912; ADAM, 1934; CHENG, 1964). To this extent the snail-host relationship has been well described. However, both early and more recent published discussions (TAYLOR & LEWIS, 1970; YONGE & THOMPSON, 1976; ELDER, 1979) have not described the pathway of the snail's proboscis into the host. Most investigators have heretofore assumed that *T. crystallina* sucks internal fluids or tissues from *L. laevigata* but have not identified the probable source of nutrients for the snail.

In the Echinodermata, nutrient transport from sites of intake or storage has been attributed to one or more of the following coelomic derivatives: the perivisceral coelom, the water vascular system, the hemal system, and the perihemal system (HYMAN, 1955; ANDERSON, 1966; FERGUSON, 1969, 1982; BINYON, 1972). To the extent that any or all of these systems are rich in nutrients, they could serve as a potential source of nutrients for *Thyca crystallina*.

In the present study we traced the route of penetration of an ectoparasitic snail's proboscis into a sea star's arm to determine which of the host's internal systems are con-

tacted. Our discovery that the snail's proboscis terminates within the perihemal system near the radial hemal strand suggests that these systems serve as a source of nutrients for *Thyca crystallina*.

MATERIALS AND METHODS

Specimens of the sea star *Linckia laevigata* were examined from collections made in May 1965, December 1970, and August 1976 on the reef flats east of the ship channel at Suva, Fiji. Thirty-two specimens of the 224 (14.3%) collected in 1965 were infected by one or more individuals of *Thyca crystallina* (a limpetlike prosobranch gastropod in the family Capulidae). Specimens were examined alive or were preserved in buffered 4% formaldehyde. Gross dissections were prepared with the aid of razor blades or a small, motor-driven, circular saw. Specimens for sections were decalcified for 2-3 days in a modified Heidenhain's Susa fixative (10 mL glacial acetic acid, 50 mL 40% formaldehyde, and 20 g trichloroacetic acid in 200 mL 70% ethyl alcohol), embedded in paraffin in a low vacuum, sectioned at 10-16 μ m, and stained with hematoxylin and Gomori's trichrome or in Cason's rapid one-step Mallory-Heidenhain.

RESULTS

We found that female specimens of *Thyca crystallina* are attached to their hosts by a circular disc as described by

ADAM (1934). We also found the males of this species attached under the shell of the females. The proboscises of the males that we examined lay freely in the space under the female shell and did not penetrate the integument of the sea star.

The attachment discs of the female *Thyca crystallina* are fused in the largest specimens to the host's integument by fibrous connective tissues. The female proboscis extends from the center of this attachment disc through a hole up to 1 mm in diameter into the tegument of the host. Gross dissections revealed that the proboscis enters the sea star's arm between the marginal dermal ossicles, progresses through the thick connective and muscular tissue of the dermis, and terminates in the middle of the ambulacral ridge (Figures 1, 2A). By angling towards the ambulacral ridge, the proboscis bypasses the perivisceral coelom, penetrates the thick body wall, and enters the ambulacral ossicles so that one or more ampullae of the host's water vascular system are lost or displaced. The only superficial evidence of the proboscis on the internal surface of the ambulacral ridge is the absence or reduction in size of 1–3 ampullae and a darkening of the ambulacral ridge in the area directly overlying the infected area; inside the ambulacral ridge this may involve the reduction in size of 2–4 ambulacral ossicles on the infected side. Discoloration results from a replacement of the ambulacral ossicle(s) lying above the proboscis by an abnormal area of hypertrophied muscular and connective tissue. These alterations in the ambulacral ossicle may be seen by comparing Figures 2B and C.

In 3 of 18 dissected specimens we observed a small hole in the depression on the aboral side of the ambulacral ridge in the middle of the discolored, infected area. In another specimen we found a small hole opening downward from the proboscis area into the ambulacral groove. Because these openings were not present in most specimens nor in any of the 12 sectioned specimens we assume they do not represent the normal condition.

The proboscis of female *Thyca crystallina* terminates in the area occupied by the hemal and perihemal systems. When uninfected areas are viewed in cross-section, these systems form a triangular area bounded on the lower side by the V-shaped radial nerve and on the upper side alternately by the lower transverse ambulacral muscle and, in the spaces between the muscles, by the radial water canal (Figure 2C). These relationships are identical in infected arms of *Linckia laevigata* proximal and distal to the infection site, *i.e.*, there is no histological evidence that the ectoparasite affects the radial systems of the host on either side of the point of infection. However, in the area penetrated by the proboscis, an extensive displacement of all systems occurs. The radial water canal is pushed to one side and the radial nerve is flattened and also pushed to the far side of the ambulacral groove; hemal tissues are often more abundant in the region near the end of the proboscis (Figure 2B). Despite the enlargement of the hemal tissue, we traced the perihemal sinus through the

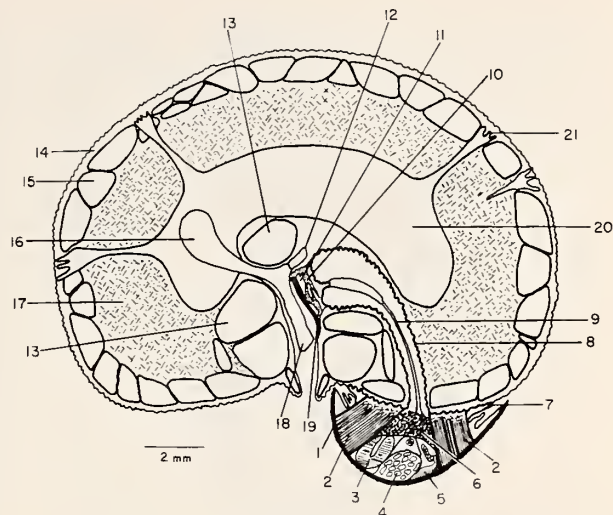


Figure 1

Schematic cross-sectional view of an arm of *Linckia laevigata* to which an ectoparasitic *Thyca crystallina* is attached. View is towards the central disc of the sea star. The snail's proboscis bypasses the perivisceral coelom, displaces 1–3 ampullae, penetrates the radial perihemal sinus, but does not interrupt the water vascular or nervous systems. *Thyca crystallina*: 1, shell; 2, columellar muscle; 3, capsule gland; 4, digestive gland; 5, nephridium; 6, salivary gland; 7, attachment disc, left anterior edge; 8, proboscis; 9, esophagus. *Linckia laevigata*: 10, hemal tissue; 11, perihemal sinus; 12, radial water canal; 13, ambulacral ossicle; 14, epidermis; 15, dermal ossicle; 16, ampulla; 17, dermis; 18, tube foot; 19, radial nerve; 20, perivisceral coelom; 21, papula.

infected area between unaltered distal and proximal regions in 11 serially sectioned specimens. In a 12th specimen the hemal tissue filled the space in front of the proboscis, thereby locally obliterating the perihemal sinuses.

The proboscis of *Thyca crystallina* consists of a sheath through which extends two salivary gland ducts and a thin-walled esophagus that terminates in a thick-walled pharyngeal mass (Figure 2D). In preserved specimens the wall of the proboscis is contracted and therefore highly folded in longitudinal sections. Except for a distinct outer epithelium, the proboscis is filled with connective tissue, blood, and blood cells. At its distal end the proboscis is folded on its outer surface, forming a groove that encloses tissues of the host (Figure 2E). This groove is continuous with the internal proboscis space through which runs the pharyngeal mass and esophagus. Inside the proboscis the groove becomes a crescentic chamber surrounding the mouth (Figure 2F); the mouth opens at the tip of the muscular pharyngeal mass. Salivary gland ducts attach to the outer surface of the muscular pharyngeal mass (Figure 2G) and empty at its distal end. Internally the pharyngeal mass is divided into a tripartite food canal which is continuous proximally with the thin-walled esophagus (Figure 2H).

Strands of host cells converge at the end of the proboscis (Figure 2B). These cells appear to originate from both the

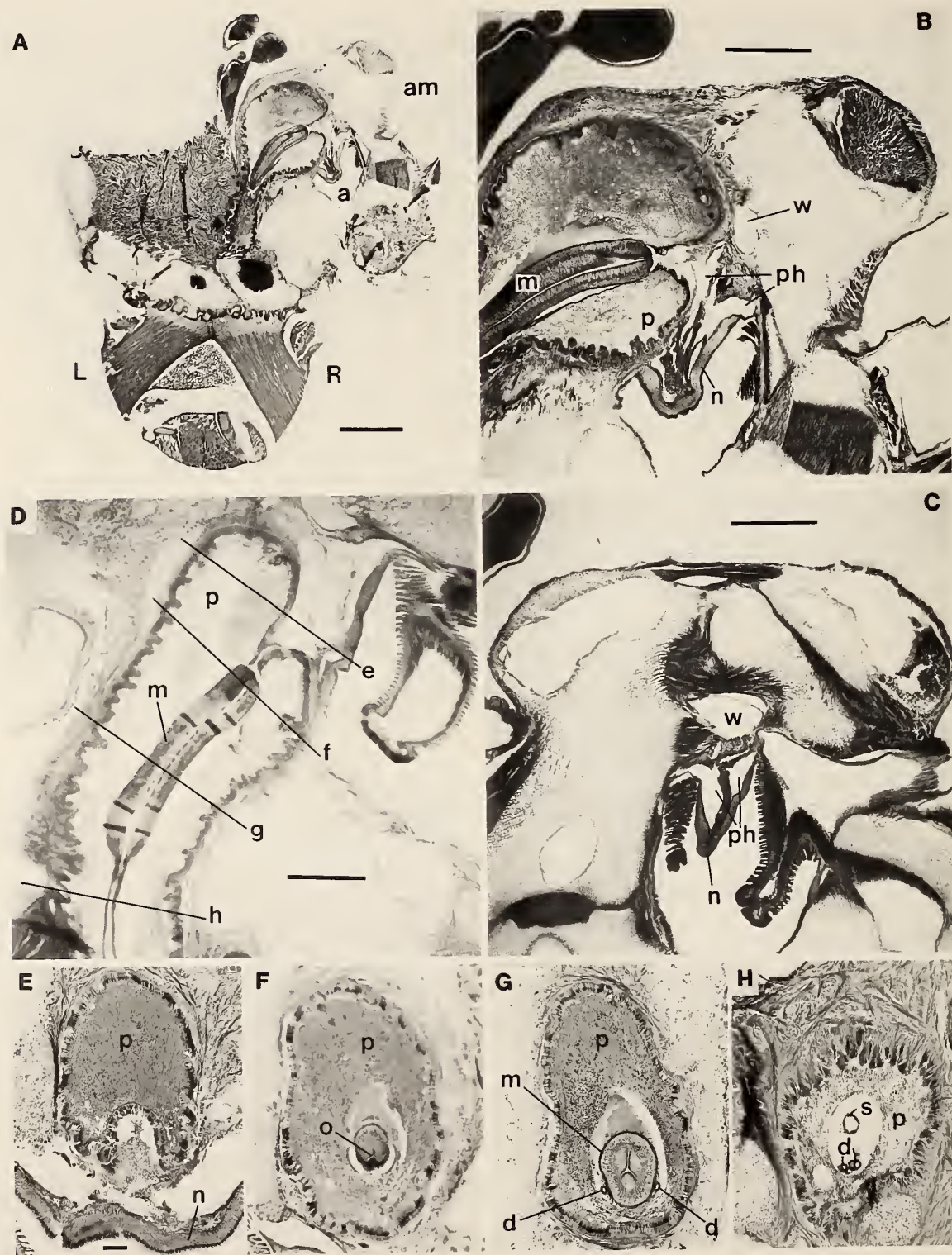


Figure 2

Photomicrographs of *Linckia laevigata* arms with and without (Figure 2C) attachment or penetration by the snail *Thyca crystallina*. A. A section through an entire snail and a fragment of a seastar arm at the level where the proboscis penetrates the perihemal sinus. The section is oriented with the ambulacrum (a) on the lower side and

host's body wall surrounding the distal end of the proboscis and from hemal tissue. The contents of the esophagus, although sparse in sectioned specimens, occasionally include nuclei of cells in addition to larger quantities of amorphous material.

DISCUSSION

The route of penetration of the proboscis of the female *Thyca crystallina* into *Linckia laevigata* suggests that sufficient food is available from some combination of hemal, perihemal, and surrounding fluids and tissues to sustain the ectoparasitic snail. This deduction is based on the fact that the proboscis of large snails cannot be withdrawn because the attachment disc is fused with the tissue of the host and the fact that the proboscis has access to no other sources of nutrients inside the host.

Alternative routes are possible but not utilized. For example, holes in the body wall occupied by papulae (Figure 1), provide a short and direct access to the perivisceral coelom, but in no specimens we examined did the proboscis of *Thyca crystallina* take this path. Instead, the proboscis bypasses the perivisceral coelom and proceeds deeper into the host. This penetration must require extensive digestion of dense dermal tissue and dissolution of calcareous ossicles. The energetic costs of creating this circuitous route to the hemal-perihemal complex ultimately must be more than offset by a flow of nutrients to the snail.

The tissues of the host at the site of penetration are not grossly injured; in fact, the opposite appears to be the case. The region at the end of the proboscis has an abundance of cells derived from the host's body wall and from hemal tissue. This suggests that the host is capable of replacing the apparent loss of cells to the snail.

Thyca crystallina presumably shuns the more spacious perivisceral coelom in favor of the relatively small hemal-perihemal complex because the latter is a more concentrated source of nutrients than is the perivisceral coelom (FERGUSON, 1982; BEIJNINK & VOOGT, 1984). This hypothesis is consistent with the observations in another asteroid, *Echinaster graminicolus*, where a radioisotopic tracer appeared within 12–24 h in the ambulacral radial hemal

tissue after injection of C^{14} -labeled amino acids (FERGUSON, 1970) or after feeding C^{14} -labeled clams or liquid glucose-amino acid medium (FERGUSON, 1984). The proteins, glycoproteins, and possibly glycolipids stored in the hemal strand of *Asterias rubens* (BEIJNINK & VOOGT, 1986) would provide a rich source of complex nutrients for *T. crystallina* if present in *Linckia laevigata*.

In addition, the snail may be obtaining soluble food produced at remote sites and transported to the snail through the perihemal sinuses. The presence of flagellated cells in the perihemal sinus (CUÉNOT, 1948; WALKER, 1979) of some asteroids provides a means of moving nutrients in solution through this system. Based on these observations, FERGUSON (1984) concluded that nutritive metabolites are translocated by some combination of movement and storage within the hemal-perihemal complex.

Given the reported anatomical relationships and the probable role of the hemal-perihemal complex in nutrient transport and storage in asteroids, the stage is set for an experimental determination of the kinds and rates of nutrient transfer through the hemal-perihemal complex of *Linckia laevigata* by using *Thyca crystallina* as a natural probe.

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LITERATURE CITED

- ADAM, W. 1934. Prosobranch parasites. *Mem. Mus. Roy. Hist. Natur. Belg.* 2(14):87–115.
 ANDERSON, J. M. 1966. Aspects of nutritional physiology. Pp. 329–357. In: R. A. Boolootian (ed.), *Physiology of Echinodermata*. Interscience Publishers: New York.
 BEIJNINK, F. B. & P. A. VOOGT. 1984. Nutrient translocation in the sea star: whole-body and microautoradiography after

ampulla (am) in the perivisceral coelom on the upper side. The left (L) and right (R) sides of the snail are indicated. B. Higher magnification of the section in Figure 2A at the distal end of the proboscis (p), which encloses the pharyngeal mass (m). The radial water canal (w), hemal tissue and associated perihemal sinuses (ph) lie above the radial nerve (n). C. A section of an uninfected part of the same seastar arm proximal to the section shown in Figures 2A, B. D. Section through the proboscis of another specimen showing the extent of the muscular pharyngeal mass (m) in the proboscis (p). Lines (e–h) indicate the level of cross sections through the proboscis of a third specimen shown in Figures 2E, F, G and H, respectively. E. Section of the proboscis (p) distal to the pharyngeal mass and mouth. The groove in the proboscis appears to surround hemal tissue lying aborally to the radial nerve (n) of the seastar. F. Section through the proboscis (p) at the point where the mouth (o) opens into the pharyngeal mass. G. Section through the middle region of the muscular pharyngeal mass (m). Ducts (d) of the salivary gland adhere closely to the outer edge of the pharyngeal mass. H. Section proximal to the pharyngeal mass showing the esophagus (s) and the ducts of the salivary gland (d). Scale bars: A, 1 mm; B–D, 0.5 mm; E, 0.1 mm (the same scale applies to F–H).

- ingestion of radiolabeled leucine and palmitic acid. *Biol. Bull.* 166:669-682.
- BEIJNINK, F. B. & P. A. VOGT. 1986. The aboral haemal system of the sea star, *Asterias rubens* (Echinodermata, Asteroidea): an ultrastructural and histochemical study. *Zoomorphology* 106:49-60.
- BINYON, J. 1972. *Physiology of echinoderms*. Pergamon Press: Oxford. 264 pp.
- CHENG, T. C. 1964. *The biology of animal parasites*. Saunders Company: Philadelphia. 727 pp.
- CUÉNOT, L. 1948. Anatomie, éthologie et systématiques des Echinodermes. In: P. P. Grassé (ed.), *Traité de zoologie* 11: 3-275. Masson: Paris.
- ELDER, H. Y. 1979. Studies on the host parasite relationship between the parasitic prosobranch *Thyca crystallina* and the asteroid starfish *Linckia laevigata*. *Jour. Zool. (Lond.)* 187: 369-391.
- FERGUSON, J. C. 1969. Feeding, digestion and nutrition in Echinodermata. Pp. 71-100. In: M. Florkin & B. T. Scheer (eds.), *Chemical zoology*. Vol. III. Echinodermata, Nematoda, and Acanthocephala. Academic Press: New York.
- FERGUSON, J. C. 1970. An autoradiographic study of the translocation and utilization of amino acids by starfish. *Biol. Bull.* 138:14-25.
- FERGUSON, J. C. 1982. Nutrient translocation. Pp. 373-393. In: M. Jangoux & J. M. Lawrence (eds.), *Echinoderm nutrition*. Balkema: Rotterdam.
- FERGUSON, J. C. 1984. Translocative functions of the enigmatic organs of starfish—the axial organ, hemal vessels, Tiedemann's bodies, and rectal caeca: an autoradiographic study. *Biol. Bull.* 166:140-155.
- HYMAN, L. 1955. *The invertebrates*. Vol. IV. Echinodermata. McGraw-Hill Book Company: New York. 763 pp.
- KOEHLER, R. & C. VANEY. 1912. Nouvelles formes de Gastéropodes ectoparasites. *Bull. Sci. Fr. Belg.* 46:191-217.
- KÜKENTHAL, W. 1897. Parasitische Schnecken. *Abh. Schneckenbergischen Ges.* 24:1-14.
- SARASIN, P. & F. SARASIN. 1887. Ueber zwei parasitische Schnecken. *Ergeb. Naturwiss. Forsch. Ceylon* 1:1-32.
- TAYLOR, J. D. & M. S. LEWIS. 1970. The flora, fauna and sediments of the marine grass beds of Mahé, Seychelles. *Jour. Natur. Hist.* 4:199-220.
- WALKER, C. W. 1979. Ultrastructure of the somatic portion of the gonads in Asteroids, with emphasis on flagellated-collar cells and nutrient transport. *Jour. Morphol.* 162:127-162.
- YONGE, C. M. & T. E. THOMPSON. 1976. *Living marine molluscs*. Collins: London. 288 pp.